

# Encapsulated nanoepigallocatechin-3-gallate and elemental selenium nanoparticles as paradigms for nanochemoprevention

Dongxu Wang<sup>1</sup>  
Ethan Will Taylor<sup>2</sup>  
Yijun Wang<sup>1</sup>  
Xiaochun Wan<sup>1</sup>  
Jinsong Zhang<sup>1</sup>

<sup>1</sup>Key Laboratory of Tea Biochemistry and Biotechnology, School of Tea and Food Science, Anhui Agricultural University, Hefei, Anhui, People's Republic of China; <sup>2</sup>Department of Nanoscience, Joint School of Nanoscience and Nanoengineering, University of North Carolina at Greensboro, Greensboro, NC, USA

**Abstract:** Chemoprevention that impedes one or more steps in carcinogenesis, via long-term administration of naturally occurring or synthetic compounds, is widely considered to be a crucial strategy for cancer control. Selenium (Se) has chemopreventive effects, but its application is limited due to a low therapeutic index as shown in numerous animal experiments. In contrast to Se, which was known for its toxicity prior to the discovery of its beneficial effects, the natural compound epigallocatechin-3-gallate (EGCG) was originally considered to be nontoxic. Due to its preventive effects on many types of cancer in various animal models, EGCG has been regarded as a prime example of a promising chemopreventive agent without major toxicity concerns. However, very recently, evidence has accumulated showing that efficacious doses of EGCG used in health promotion may not be far from its toxic dose level. Therefore, both Se and EGCG need to be modified by novel pharmaceutical technologies to attain enhanced efficacy and/or reduced toxicity. Nanotechnology may be one of these technologies. In support of this hypothesis, the characteristics of polylactic acid and polyethylene glycol-encapsulated nano-EGCG and elemental Se nanoparticles dispersed by bovine serum albumin are reviewed in this article. Encapsulation of EGCG to form nano-EGCG leads to its enhanced stability in plasma and remarkably superior chemopreventive effects, with more than tenfold dose advantages in inducing apoptosis and inhibition of both angiogenesis and tumor growth. Se at nanoparticle size ("Nano-Se"), compared with Se compounds commonly used in dietary supplements, has significantly lower toxicity, without compromising its ability to upregulate selenoenzymes at nutritional levels and induce phase II enzymes at supranutritional levels.

**Keywords:** epigallocatechin-3-gallate, chemoprevention, nanoparticles, selenium

## Chemoprevention

Chemoprevention is defined as the use of compounds to inhibit the development of cancer, either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells in which such damage has already occurred.<sup>1</sup> The expanded definition of chemoprevention is: through the use of natural or synthetic substances, to reverse, suppress, and prevent either the initial phase of carcinogenesis or the progression of neoplastic cells to cancer.<sup>2</sup> Among many diverse chemopreventive agents, epigallocatechin-3-gallate (EGCG) and various forms of selenium (Se) have been extensively investigated.<sup>3,4</sup>

## EGCG

Tea is one of the most widely consumed beverages worldwide. Green tea contains large quantities of biologically active catechins, which have been identified as

Correspondence: Jinsong Zhang  
Key Laboratory of Tea Biochemistry and Biotechnology, School of Tea and Food Science, Anhui Agricultural University, 230036 Hefei, Anhui, People's Republic of China  
Tel +86 551 578 6283  
Fax +86 551 578 6283  
Email zjs@ahau.edu.cn

important dietary factors for health promotion over the past two decades.<sup>5</sup> Among the six kinds of catechins in green tea, EGCG accounts for half or more of total catechin in green tea.<sup>6</sup>

## Efficacious and toxic doses of EGCG

The chemopreventive effects of EGCG have been confirmed in at least 13 human or animal organs, including the esophagus, stomach, lung, small intestine, large intestine, colon, skin, liver, bladder, prostate, pancreas, mammary glands, and oral cavity.<sup>7</sup> Most of the studies used the regimen of adding EGCG or green tea extract with EGCG as the major component to drinking water.<sup>8–13</sup> As a prominent chemopreventive agent, EGCG must be amenable to prolonged ingestion at levels in excess of normal dietary intake without inducing adverse effects. Because the potential toxicity related to such a regimen has not been investigated, it remains uncertain whether the efficacious doses for chemoprevention are really as far away from the toxic doses as have been superficially inferred.

However, EGCG administration via diet or intraperitoneal (IP) injection has been reported to be associated with various adverse effects. Mice consuming a diet with 1% EGCG for 6 weeks exhibited elevated splenocyte and macrophage proinflammatory markers such as tumor necrosis factor- $\alpha$ , interleukin-6, interleukin-1 $\beta$ , and prostaglandin E<sub>2</sub> and disturbed immune cell populations.<sup>14</sup> A single IP administration of 100 mg/kg EGCG to mice can generate hepatotoxicity, whereas 150 mg/kg results in 100% mortality within 24 hours.<sup>15</sup> Furthermore, a causal association between the consumption of green tea extract and liver damage has recently been established in humans.<sup>16</sup> Thirty-four cases of hepatitis following the consumption

of green tea extract for the purpose of obesity control have been documented.<sup>16</sup> Upon liver histological examination, inflammatory reactions, cholestasis, steatosis, and necrosis were noted. A positive dechallenge was reported in 29 cases, and a positive rechallenge occurred in seven cases.<sup>16</sup> The mechanism of EGCG toxicity has been ascribed to its pro-oxidant action, because oxidative stress-associated biomarkers, including hepatic malonyldialdehyde, 4-hydroxynonenal, metallothionein, and phosphorylated histone 2AX, substantially increase in EGCG-intoxicated mice.<sup>17</sup> Tea can contain pesticide residues that might cause adverse effects, including hepatotoxicity. Therefore, some cases of tea-related hepatotoxicity may be ascribed to the presence of pesticide residues in tea. However, the published data showing that EGCG is able to cause adverse effects in experimental animals were obtained by using purified EGCG.

In addition to its cancer-preventive effect, EGCG has potential in the prevention of obesity, diabetes, and neurodegenerative diseases. Herein we listed some literature-reported beneficial doses administered through diet or the IP route to obtain a window concept of efficacious doses and toxic doses (Table 1). In the studies involving cancer, 0.5%–1% EGCG in the diet was used for 7–24 weeks;<sup>18,19</sup> 50–60 mg/kg EGCG was IP administered for 2–23 weeks.<sup>20,21</sup> In the studies involving type 2 diabetes mellitus and obesity, 0.32%–1% EGCG in the diet was used for 4–16 weeks.<sup>22–25</sup> In the studies involving liver and brain protection, 50–75 mg/kg EGCG was IP administered for 1–56 days.<sup>14,26,27</sup> Based on the evidence that both 1% EGCG in the diet for 6 weeks of administration and a single IP injection of 100 mg/kg EGCG have adverse effects on mice, it seems obvious that the efficacious doses of EGCG including chemopreventive doses are not far from

**Table 1** Efficacious doses and toxic doses of epigallocatechin-3-gallate in mice

Delivery route	Times of administration	Outcomes	References
0.32% in diet	16 weeks	Inhibiting obesity and fatty liver	23,24
0.5% in diet	7 weeks	Inhibiting tumor development	18
1% in diet	24 weeks	Not inhibiting B( $\alpha$ )P-promoted tumorigenesis	19
1% in diet	7 weeks	Alleviating type 2 diabetes mellitus	25
1% in diet	4 weeks	Inhibiting obesity	22,24
1% in diet	6 weeks	Proinflammatory responses	14
IP 50 mg/kg	2 weeks	Reducing angiogenesis	20
IP 50 mg/kg	1 day	Reducing brain damage	26
IP 50 mg/kg	8 weeks	Reducing liver fibrosis	27
IP 60 mg/kg	23 weeks	Inhibiting 1,2-DMH-promoted tumorigenesis	21
IP 50–75 mg/kg	3 days	Preventing acute hepatotoxicity	14
IP 100 mg/kg	1 day	Hepatotoxicity	15
IP 150 mg/kg	1 day	Died within 24 hours	15

**Abbreviations:** 1,2-DMH, 1,2-dimethylhydrazine; B( $\alpha$ )P, benzo( $\alpha$ )pyrene; IP, intraperitoneal.

its toxic dose levels; furthermore, some efficacious doses actually overlap with the toxic doses.

## Intervention time of EGCG in chemoprevention

In addition to the aforementioned dose concerns, results obtained regarding the optimal intervention time for EGCG chemoprevention are not particularly promising either. In a transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which closely emulates human disease, it was found that an intervention starting at the age of 8 weeks by adding green tea polyphenol (GTP), with EGCG as the major component to drinking water at a concentration of 0.1% (w/v), generated pronounced chemopreventive efficacy.<sup>28</sup> Specifically, the GTP treatment reduced the cancer incidence in TRAMP mice from 100% to 35% and the cancer metastasis to lymph and liver to null from 95% and 65%, respectively. Without the GTP treatment, the TRAMP mice had enlarged prostate and genitourinary weight (4.6- and 8.3-fold compared with the nontransgenic mice, respectively), whereas the GTP treatment decreased prostate and genitourinary hyperplasia by 64% and 72%, respectively. The GTP treatment significantly increased median life expectancy of TRAMP mice from 42 weeks to 68 weeks. In the serum of TRAMP mice, elevated insulin-like growth factor-1 and vascular endothelial growth factor were significantly reversed by the GTP treatment. In the prostate tissue of TRAMP mice, several key proliferation-associated signaling proteins and metastasis-related proteins were substantially suppressed by the GTP treatment.<sup>28,29</sup> These impressive experimental results suggest that GTP or EGCG has tremendous potential for prostate cancer prevention.

The impact of GTP intervention time on prostate cancer prevention in TRAMP mice was further investigated by the same team. Significantly, unlike the promising results obtained when GTP was initiated at the age of 8 weeks, when treatment started at 18 weeks the preventive effect was largely compromised, whereas when begun at the age of 28 weeks the preventive effect disappeared almost completely.<sup>30</sup> The median life span of TRAMP mice is 42 weeks. Given that the human median life span is 70 years, accordingly (if the effect in humans was analogous), someone starting to drink green tea at 13 years old (equivalent to the 8th week of TRAMP mice) might gain a pronounced chemopreventive effect, whereas starting at 30 years old (equivalent to the 18th week of TRAMP mice) might have only a weak effect on cancer development. Thus, the promising chemopreventive effects of GTP might be largely limited to earlier intervention for

adolescents, who nowadays prefer carbonated drinks rather than tea in many parts of the world. For adults who are willing to accept chemopreventive practices, the chemopreventive efficacy of EGCG needs to be enhanced.

## Encapsulated nano-EGCG for chemoprevention

Typically, but not exclusively, nanoscience investigates objects in the range of 1–100 nm.<sup>31</sup> As applied to biology, this field has led to the advent of nanomedicine, which has many facets, one of the most important of which is the nanofabrication of drugs in nanoparticle-based drug-delivery systems.<sup>32–34</sup> One advantage of this technology is that drugs included in nanoformulations can be protected from the destructive action of external media.<sup>35</sup> In addition, it is now well established that drugs encapsulated in nanoparticles exhibit distinct pharmacokinetic and pharmacodynamic profiles as compared with the nonencapsulated free drugs.<sup>36,37</sup>

“Nanochemoprevention”, a term coined by Siddiqui and Mukhtar<sup>38</sup> very recently, involves the utilization of nanotechnology to improve the pharmacokinetic and pharmacodynamic profiles of chemopreventive agents. For example, curcumin is a widely studied phytochemical with chemopreventive potential. Encapsulated nanocurcumin manifests enhanced cellular uptake and cytotoxicity *in vitro*, as well as superior bioavailability and anticancer activity *in vivo* over nonencapsulated free curcumin.<sup>39–41</sup> EGCG encapsulated in lipid nanocapsules exhibited a stable status without degradation in the aqueous phase over 4 weeks, whereas free EGCG totally degraded within 4 hours.<sup>42</sup> When EGCG is encapsulated in chitosan, its bioavailability significantly increases compared with nonencapsulated free EGCG. Specifically, oral administration of chitosan-encapsulated nano-EGCG enhanced intestinal absorption by a factor of 1.8 relative to free EGCG and enhanced the plasma exposure of total EGCG by a factor of 1.5 relative to free EGCG.<sup>43,44</sup> Polylactic acid (PLA) and polyethylene glycol (PEG) are biologically inert and completely biocompatible without toxicity or antigenic reactions.<sup>45</sup> EGCG can be encapsulated in PLA–PEG nanoparticles<sup>46</sup> whose average size is 260 nm, as shown in supplementary figure S1–2 in Siddiqui et al.<sup>46</sup> The biological activities of PLA–PEG-encapsulated nano-EGCG versus nonencapsulated free EGCG have been compared in term of apoptosis induction, inhibition of angiogenesis and tumor growth, and EGCG retention in blood after IP administration, as summarized in Table 2.<sup>46</sup> Overall, PLA–PEG-encapsulated nano-EGCG, compared with the nonencapsulated free EGCG, is resistant

**Table 2** Comparison of biological activities between encapsulated nanoepigallocatechin-3-gallate (EGCG) and nonencapsulated free EGCG

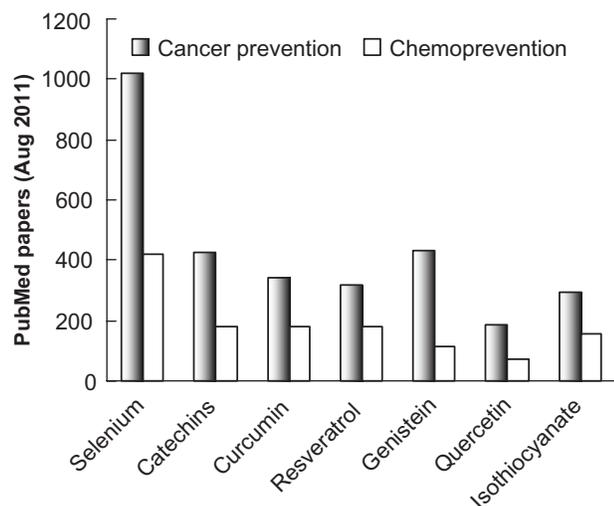
Biomarkers	Encapsulated nano-EGCG	Nonencapsulated free EGCG
IC <sub>50</sub> of PCa PC3 cells	3.74 µmol/L	43.6 µmol/L
Doses needed to generate 72% apoptosis in PCa PC3 cells	2.7 µmol/L	40 µmol/L
Inhibiting colonies formation of PCa PC3 cells	5.5 µmol/L inhibited 90%	20 µmol/L inhibited 10%
Bax/Bcl-2 ratio of PCa PC3 cells	2 at 1.4 µmol/L	0.5 at 20 µmol/L
Inhibition of FGF-promoted angiogenesis in vitro	3 µg/CAM generated 57% inhibition	30 µg/CAM generated 35% inhibition
Suppressing tumor growth in mice inoculated with androgen-responsive 22Rv1 cells after 7 weeks of EGCG administration	IP 0.1 mg/mouse inhibited 50% as compared with tumor control	IP 1 mg/mouse inhibited 50% as compared with tumor control
Serum PSA of mice inoculated with androgen-responsive 22Rv1 cells after 7 weeks of EGCG administration	10% of tumor control/IP 0.1 mg/mouse	75% of tumor control/IP 1 mg/mouse
EGCG degradation in plasma of mice	EGCG existed in plasma after 4 hours/IP 0.1 mg/mouse	EGCG disappeared from plasma after 4 hours/IP 1 mg/mouse

**Abbreviations:** CAM, chick chorioallantoic membrane; FGF, fibroblast growth factor; IC<sub>50</sub>, the half maximal inhibitory concentration; IP, intraperitoneal; PCa, prostate cancer; PSA, prostate-specific antigen.

to degradation in blood and produces remarkably superior chemopreventive effects, with an over tenfold dose advantage in inducing apoptosis and inhibiting angiogenesis and tumor growth. Together, these studies reveal that nanoparticle-mediated delivery of EGCG could serve as a basis for enhancing the bioavailability of EGCG.

## Selenium

Although phytochemicals including EGCG have received considerable attention for their cancer-preventive effects, the most extensively investigated chemopreventive agent is Se, as indicated in Figure 1, which depicts the search results for the number of papers in PubMed using the keywords “cancer prevention” or “chemoprevention” along with the specific chemopreventive agent.

**Figure 1** Number of publications on cancer prevention by chemopreventive agents.

## Se-dependent selenoproteins and cancer prevention

Se is capable of exerting multiple actions on the physiological system by modifying the expression of 25 human selenoproteins, whose synthesis is dependent upon the incorporation of the 21st genetically encoded protein amino acid, selenocysteine.<sup>47–49</sup> Most of the selenoproteins, such as selenoenzymes of glutathione peroxidases (GPx) and thioredoxin reductases (TrxR), take part in antioxidant defense.<sup>50–52</sup> Activities of selenoenzymes are affected by Se at nutritional levels; therefore, Se deficiency leads to reduced activities of selenoenzymes.<sup>53,54</sup> Transgenic mice whose selenoprotein synthesis is disrupted are predisposed to precancerous changes.<sup>55,56</sup> Human epidemiological studies have found an inverse relationship between Se status and cancer risk.<sup>4,57</sup> In SELECT (the Selenium and Vitamin E Cancer Prevention Trial), the participants had optimal Se status; thus, Se supplementation at nutritional level had no effect on cancer risk, whereas the participants with low Se status showed reduced cancer risk after Se supplementation at nutritional levels.<sup>58,59</sup> These results suggest that Se at nutritional levels has a cancer-prevention effect via enhancing the expression of selenoproteins in those subjects with suboptimal Se status.

## Se-induced phase II enzymes in cancer prevention

Phase II enzymes such as quinone reductase and glutathione S-transferase (GST) are a class of inducible enzymes that are upregulated in response to toxic insults.<sup>60</sup> Upregulation of phase II enzymes has been implicated in the detoxification

of numerous oxidative and electrophilic species during xenobiotic metabolism.<sup>61</sup> The cancer-preventive effect of many chemopreventive agents, including EGCG, sulforaphane, curcumin, and resveratrol, is associated with the upregulation of phase II enzymes.<sup>62–66</sup> Among various phase II enzymes, GST plays an important role in cellular protection against carcinogens by conjugating their electrophilic metabolites with GSH.<sup>67,68</sup> Evidence suggests that the level of GST expression is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals; hence, the induction of GST by chemopreventive agents enables experimental animals to tolerate exposure to carcinogens.<sup>69</sup> Enhanced GST expression can limit tumor development.<sup>70</sup> Many Se compounds can oxidize thiols, consequently producing superoxide and other reactive oxygen species (ROS).<sup>71,72</sup> Modest amounts of ROS promote the translocation of the transcription factor Nrf2 into the nucleus, where Nrf2 binds to the antioxidant response element in phase II enzyme genes to activate the transcription of phase II enzyme mRNAs.<sup>73</sup> Xiao and Parkin<sup>74</sup> found that 16 Se compounds were able to increase quinone reductase activity, and seven of them also increased GST activity in murine hepatoma cells. Se at supranutritional levels, which are roughly ten- to 30-fold higher than nutritional levels, is capable of inducing phase II enzymes and exhibits powerful chemopreventive effects.<sup>75–79</sup> Thus, the chemopreventive effects of Se at supranutritional levels are associated with the induction of phase II enzymes.

## Se-mediated cytotoxicity and cancer prevention

The ROS that originate from Se-promoted thiol oxidation, if in sufficient quantity, will result in intracellular and extracellular oxidative stress, leading to cytotoxicity.<sup>80,81</sup> The cytotoxic effects of Se may partially account for their chemopreventive activity.<sup>82–84</sup> However, the general therapeutic utility of this mechanism is questionable and should be approached with caution. It is known that inhibition of TrxR results in enhanced selenite cytotoxicity and that cells overexpressing TrxR1 are significantly more resistant to selenite cytotoxicity than control cells.<sup>85,86</sup> TrxR1 has been shown to be upregulated in various cancer cells; thus, cancer cells are likely to be more resistant than normal cells to Se cytotoxicity. Drug-resistant tumor cells with high intracellular GSH exhibit a high degree of sensitivity to selenite cytotoxicity, whereas normal cells with high intracellular GSH would be more sensitive to Se cytotoxicity than some types of cancer cells with low

intracellular GSH.<sup>87,88</sup> Normal cells possess functional p53, which is mutated in most cancer cells. It has been shown that p53 can enhance the cytotoxicity of Se, suggesting that normal cells may be more sensitive to Se cytotoxicity than p53-mutated cancer cells.<sup>89–92</sup> Indeed, the cytotoxic effects of both inorganic and organic Se compounds were more potent in normal hepatocytes as compared with hepatic carcinoma cells, and nontumorigenic prostate cells are highly sensitive to Se toxicity as compared with prostate cancer cells at physiologically relevant concentrations.<sup>93,94</sup> For Se-induced cytotoxicity to be able to operate as a chemopreventive mechanism, Se toxicity would appear to be unavoidable. Therefore, Se-dependent selenoproteins and Se-induced phase II enzyme mechanisms, which are not associated with evoked toxicity, become more attractive to explain the cancer-preventive effects of Se, whereas the “enhanced cytotoxicity” mechanism ought to be limited to Se-sensitive cancer cells whose proliferation can be effectively suppressed by Se at safe doses.

## Preparation of elemental Se nanoparticles

A decade ago, elemental Se in the redox state of zero was considered to be biologically inert.<sup>95</sup> Indeed, red elemental Se, formed in the redox system of selenite and GSH, is unstable and can further aggregate into gray or black elemental Se if there are no controlling factors in the redox system, leading to the disappearance of bioactivities.<sup>96</sup> We reported in 2001 that the presence of proteins such as bovine serum albumin (BSA) in the redox system at a tenfold excess by mass relative to Se can control the aggregation of elemental Se atoms; the resultant Se particles, referred to as Nano-Se, fall into a size distribution of 20–60 nm, with an average size of 36 nm.<sup>96</sup> Consistent with this finding, Mishra et al<sup>97</sup> demonstrated the formation of BSA-dispersed Se nanoparticles when selenourea was oxidized into elemental Se. Dobias et al<sup>98</sup> recently showed that some particular proteins, such as alcohol dehydrogenase, can specifically bind to Se nanoparticles, resulting in a narrower size distribution. In addition to protein, polysaccharides have been revealed to be effective dispersants for controlling the formation of Se nanoparticles.<sup>99,100</sup> The formation of Se nanoparticles is not limited to *in vitro* conditions, as some strains of micro-organisms have the capacity of reducing selenite into Se nanoparticles.<sup>101–103</sup>

The bioactivities and toxicities of inorganic sodium selenite, organic selenomethionine (SeMet), and Se-methylselenocysteine (SeMSC) have been extensively investigated.

Based on the preceding review, the optimal form of Se for nutritional supplementation and cancer prevention would be expected to have distinctly low toxicity and to possess good bioactivities in terms of upregulating selenoenzymes at nutritional levels and inducing phase II enzymes at supra-nutritional levels.

## Comparison of bioactivities and toxicities between inorganic Se and Nano-Se

Sodium selenite has been used in livestock and humans to prevent Se-deficiency disorders. Usually, it is used as a reference Se compound in the studies of Se bioavailability and toxicity. In HepG2 cells, although selenite dose-dependently increased GPx and PHGPx activities ( $R^2 = 0.9881$  and  $0.9956$ , respectively), there were no significant differences in elevating these selenoenzyme activities between selenite and Nano-Se at the same doses, based on total Se content.<sup>96</sup> When Nano-Se and selenite were added to a Se-deficient diet at a level of 0.1 ppm Se for Se supplementation in rats, selenite significantly increased hepatic Se by 8.8-fold and hepatic GPx activity by 48.8-fold. There were no significant differences in these biomarkers between selenite and Nano-Se.<sup>96</sup> These *in vitro* and *in vivo* results demonstrate that the bioavailability of the two Se sources is equal.

Selenite toxicity is associated with the interaction of selenite with GSH to form reactive selenotrisulfides, leading to the production of ROS.<sup>80</sup> Selenite is one order of magnitude more effective than Nano-Se in oxidizing GSH, suggesting that the cytotoxic effect of selenite but not Nano-Se may

be enhanced by extracellular GSH.<sup>96</sup> Indeed, exposure of HepG2 cells to the cotreatment of nontoxic Nano-Se and GSH reveals no cytotoxicity, whereas exposure of HepG2 cells to the cotreatment of an otherwise nontoxic dose of selenite, but in the presence of GSH, produced significant cytotoxicity.<sup>96</sup> According to this evidence, it is anticipated that in tissues where extracellular GSH is elevated, enhanced cytotoxicity will be much more likely to occur for selenite than for Nano-Se. Consequently, selenite would be more toxic than Nano-Se *in vivo*. Indeed, the oral acute toxicity of selenite was 7.2-fold that of Nano-Se, according to the medium lethal dose ( $LD_{50}$ ) values obtained from mice.<sup>96</sup> The US National Research Council recommends growth inhibition as the best indicator of Se toxicity.<sup>104</sup> The major target of Se toxicity is liver tissue.<sup>105</sup> In a short-term toxicity study, mice were orally administered saline as control, Nano-Se, and selenite at 4 mg Se/kg for 4 weeks. Body weight in the selenite group was significantly suppressed by 30%, whereas body weight in the Nano-Se group remained not significantly different from the control.<sup>106</sup> At the end of the experiments, selenite caused prominent liver injury, whereas the hepatic architecture in the Nano-Se group remained unaltered.<sup>106</sup> Furthermore, in a subchronic toxicity study in which rats were fed with diets containing 0 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm Se for 13 weeks, Nano-Se unequivocally manifested lower toxicity compared with either inorganic selenite or naturally occurring Se-enriched soy protein (high-Se protein) in all observed biomarkers, including growth inhibition, hematology, clinical chemistry, relative organ weights, and histopathology parameters (Table 3).<sup>107</sup>

**Table 3** Subchronic toxicity of selenium (Se) compounds in rats

Biomarkers	Se (ppm) in diet	Nano-Se	Selenite	High-Se protein
NOAEL (ppm)		3	2	2
Growth retardation	3	Nano-Se < selenite and high-Se protein		
BWL	3	Nano-Se < selenite and high-Se protein		
	4	Nano-Se < selenite and high-Se protein		
Reduction of erythrocyte, hemoglobin, platelet counts	4	Not significantly	Significantly	Significantly
Spleen enlargement and liver atrophy	3	Not significantly	Not significantly	Significantly
	4	Not significantly	Significantly	Significantly
Mottled liver surface	4	Nano-Se < selenite and high-Se protein		
	5	Nano-Se < selenite and high-Se protein		
Degeneration of liver cells	3	None	Existence	Existence
Patchy necrosis	5	None	Existence	Existence
Increase of ALT activity	4	Not significantly	Significantly	Not significantly
Increase of AST activity	4	Not significantly	Significantly	Not significantly
	5	Not significantly	Significantly	Significantly
Increase of TP activity	5	Not significantly	Significantly	Significantly
Increase of ALB activity	5	Not significantly	Significantly	Significantly

**Abbreviations:** ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BWL, body weight loss; NOAEL, the no-observed-adverse-effect level; TP, total protein.

## Comparison of bioactivities and toxicities between organic Se and Nano-Se

SeMet is the predominant chemical form of Se in foodstuffs. Numerous experimental studies have suggested that SeMet has excellent bioavailability and lower toxicity, as compared with selenite.<sup>108</sup> Because Nano-Se has lower toxicity profiles compared with the high-Se protein whose Se constitution would be largely taken up by SeMet, it is warranted to make direct and comprehensive comparisons between Nano-Se and SeMet in terms of bioactivities and toxicity.<sup>107</sup> SeMet and Nano-Se were orally administered to Se-deficient mice daily for 7 days at two nutritional doses to compare bioavailability, and at a supranutritional dose to evaluate phase II enzyme induction. Although SeMet has been considered as a good Se source with excellent bioavailability, at the two tested nutritional doses, SeMet and Nano-Se equally increased tissue Se levels and the activities of GPx and TrxR.<sup>109</sup> Significant differences between the two Se sources were found at the supranutritional dose; SeMet increased Se levels more efficiently than Nano-Se in all measured tissues, including the liver, kidney, and blood.<sup>109</sup> However, the high retention of Se in the liver subjected to SeMet did not guarantee that SeMet could increase hepatic GST activity; in contrast, Nano-Se, which provided less Se to the liver compared with SeMet, generated a significant induction of hepatic GST compared with either the control group or the SeMet group.<sup>109</sup> Se sequestration in protein via the nonspecific replacement of methionine using SeMet can readily explain such a paradoxical result.<sup>110</sup> Excess substitution of methionine residues by SeMet may alter physiochemical properties of some structural proteins and reduce the accumulation of active Se species that exert anticancer actions.<sup>110</sup>

Thus, the high Se accumulation deposited by SeMet at supranutritional levels cannot necessarily be considered as a merit; in contrast, it reduces the chemopreventive potential of SeMet, as evidenced by the GST induction, and increases the risk of SeMet toxicity. This interpretation is supported by the following results: (1) the acute oral toxicity of SeMet was 3.6-fold that of Nano-Se according to the LD<sub>50</sub> values obtained from mice;<sup>109</sup> (2) following administration of a single oral dose of 10 mg Se/kg to mice, after 12 hours, Nano-Se did not significantly elevate serum liver enzymes, but SeMet significantly increased serum alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase activities by fourfold to 25-fold relative to the control;<sup>109</sup> and (3) following repetitive daily oral administration of 5 mg Se/kg/day to mice for 7 days, SeMet exhibited significantly higher toxicity than Nano-Se in terms of growth suppression and liver injury.<sup>109</sup>

SeMSC is considered to be one of the most effective Se compounds for chemoprevention, but unfortunately its systemic toxicities are high as well.<sup>112–114</sup> Zhang et al<sup>111</sup> have demonstrated that SeMSC and Nano-Se have equal bioavailability at nutritional doses. Although the GST induction efficacy of the two Se sources was similar at supranutritional doses, SeMSC had a greater tendency toward Se toxicity.<sup>111</sup> This is evidenced by: (1) the acute oral toxicity of SeMSC was 6.3-fold that of Nano-Se according to the LD<sub>50</sub> values obtained from mice;<sup>111</sup> (2) following administration of a single oral dose of 10 mg Se/kg to mice, after 12 hours, serum alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase activities were all significantly higher in SeMSC-treated mice than in Nano-Se-treated mice;<sup>111</sup> and (3) following repetitive daily oral administration of 10 mg Se/kg/day to mice for 7 days, SeMSC resulted in 80% mortality, whereas Nano-Se resulted in only 10% mortality.<sup>111</sup>

At supranutritional levels, Wang et al<sup>109</sup> and Zhang et al<sup>111</sup> found that SeMSC, as with SeMet, increased Se levels more efficiently than Nano-Se in all measured tissues. High Se accumulation in SeMet-treated mice can be attributed to Se sequestration via nonspecific substitution of SeMet for methionine in proteins. Obviously, such a mechanism cannot apply in the case of high Se accumulation produced by supranutritional SeMSC; however, the significant differences in tissue Se retention between SeMSC and Nano-Se may be affected by the pore size of vessels. The pore sizes of normal vessels are 2–6 nm, so the entry of SeMSC molecules at approximately 1 nm appears not to be limited, whereas the entry of Se nanoparticles with an average size of 36 nm (20–60 nm) should be affected by vessel pore sizes. To support this hypothesis, ie, to observe the impact of size on Se accumulation, we prepared two kinds of Nano-Se with different size distributions, based on the principle that, during their preparation, a higher BSA concentration generates smaller Se nanoparticles.<sup>115,116</sup> As expected, we found that Se accumulation at supranutritional levels was size dependent, such that small size led to high Se retention.<sup>116</sup> It is worth noting that size-dependent Se accumulation has an important implication in explaining the low toxicity of Nano-Se. Cells may change to passive absorption of Se at near-toxic supranutritional levels after the most fundamental physiological needs of a cell for selenoenzyme synthesis have been fully met. Under such conditions, large size would constitute a barrier for Se nanoparticles to enter into cells.<sup>117</sup>

Therefore, the significantly reduced Se accumulation in tissues subjected to Nano-Se at supranutritional levels as compared with SeMet or SeMSC may effectively prevent

Se toxicity.<sup>109,111</sup> In addition, although Se nanoparticles show reduced Se retention in normal tissues as compared with SeMet and SeMSC, Se nanoparticles may show enhanced Se permeation and retention in tumor tissues, because tumor blood vessels possess large pores with a size distribution ranging from 100 nm to 800 nm, in stark contrast to small pores of 2–6 nm in the vessels of healthy tissues.<sup>118</sup> This would confer a unique “targeting” advantage to Nano-Se, unavailable with other forms of Se. Recently, Sommer et al<sup>119</sup> demonstrated that pulsed red laser light can force cancer cells to take up cytotoxic drugs, including EGCG, via nanoscopic interfacial water layers in cells, thereby resulting in enhanced cytotoxicity. Because nanoparticles have inherent characteristics of enhanced permeation and retention in tumor tissues,<sup>118</sup> with the auxiliary effect of pulsed red laser light, the targeting advantage of Nano-Se and nano-EGCG would likely be further enhanced.

## Conclusions and future prospectives

EGCG is a naturally occurring chemopreventive agent. PLA-PEG-encapsulated nano-EGCG, compared with nonencapsulated free EGCG, is resistant to degradation in blood and produces remarkably superior chemopreventive effects, with over a tenfold dose advantage in inducing apoptosis, inhibiting angiogenesis and tumor growth. So Nano-EGCG provides a paradigm for the use of nanoparticle-mediated delivery to enhance bioavailability. Se is a chemopreventive agent with a narrow margin between toxic amounts and the amounts needed for dietary requirements or therapeutic effects, ie, a low therapeutic ratio. Compared with selenite, SeMet, and SeMSC, Nano-Se has significantly lower toxicity, without compromising the important therapeutic capacities of increasing the activities of selenoenzymes and phase II enzymes. The safety margin and potential toxic effects of Se are important considerations for its role in supplementation. Therefore, Nano-Se can be considered as a novel chemoprevention agent with reduced risk of Se toxicity. Nanotechnology holds promise for chemoprevention, because anticancer nutrients fabricated at the nanometer scale exhibit drastically altered bioactivities and toxicity. Sustained exploration of the nanochemoprevention concept may lead to exciting new horizons in the discovery of novel chemopreventive agents, with an expanded window between efficacious doses and toxic doses.

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## Disclosure

No conflict of interest was reported by the authors of this article.

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